Notes on Certain Aspects of the Development of Trypanosoma gambiense *in* Glossina palpalis.

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In the course of an attempt to obtain an insight into the details of the life-cycle of *Trypanosoma gambiense* in *Glossina palpalis*, certain experiments were undertaken involving the feeding of a relatively large number of flies under closely observed conditions. Although primarily undertaken with a view to the morphology and development of the parasite, they have a bearing on the general relation between the trypanosomes and the Glossina that is of some interest.

The present account deals with the infections produced in the flies as a whole; the morphological results will be considered in detail elsewhere. I must point out that the experiments in question are not concerned with actual transmissions of *T. gambiense* from an infected to a clean host, but with the number of flies in which the trypanosomes will develop. That flies harbouring trypanosomes are infective from about the 26th day onwards has been shown over and over again; it was therefore considered to be a wanton waste of life to allow every cage kept beyond the 30th day to infect a clean animal. Late cages were usually fed on cock's blood after the 24th day. A small proportion were actually tested and an infection was invariably produced if the box contained flies showing trypanosomes.

There is no evidence to show that a trypanosome-infection once established in the fly is ever got rid of subsequently. *T. gambiense* may be held to be established if the gut shows trypanosomes after the 5th day in flies which have had *at least one feed of clean blood* subsequent to the infecting feed.

This last statement bears on a point of some importance; it has been found during the course of these experiments that flies allowed to have one infected feed and then starved absolutely, when dissected between the 6th and the 12th day, show an extraordinary number of individuals in which trypanosomes are to be found. Flies starved in this way rarely live beyond the 12th or 13th day. These experiments will be referred to as starvation experiments.

Of 103 flies so treated and fed (for one infecting feed only) in groups on different monkeys infected with *T. gambiense*, 22 showed trypanosomes

between the 6th and 12th day, that is to say 21·3 per cent. of the flies harboured trypanosomes. Six flies of the total 22 showed trypanosomes only in the sucking stomach, or crop, as this organ should perhaps be more appropriately called, 13 showed a well-established infection in the gut and three showed trypanosomes in both situations. It is perhaps advisable to neglect the six flies in which the parasite was only present in the crop, although a certain amount of development may go on in this organ; the percentage thus obtained is still very high, namely 15·5.

Monkeys infected with *T. gambiense*, and probably most other animals with trypanosomes in their blood, have negative periods, that is to say, periods during which they do not infect flies. A number of the experiments have shown that trypanosomes may be found by microscopic examination, although the blood is not infective to flies. It is interesting to note that such negative periods appear to be negative in starved as well as in fed flies. This relation is, however, not yet sufficiently worked out. In the starvation experiments the microscopic appearances do not, so far as I have yet seen, show any distinction from those to be observed in established infections in fed flies of the corresponding ages.

The crucial moment in the cycle appears to be the first feed of clean blood subsequent to the infecting feed. It is not evident if this clearing out of the trypanosomes by the clean feed is a purely mechanical action due to the flooding of the gut or is a result of the general change of condition thus brought about.

In any case the number of flies containing trypanosomes obtained in starvation experiments during periods when the vertebrate is in the infective condition would give the maximum register of the potential infectivity of that individual strain to fly. The actual number of flies containing trypanosomes from parallel experiments, which were, however, subsequently fed, would give an indication of the additional inhibiting power of the fly under ordinary conditions whatever the cause to which the inhibition may be due. Experiments of this type were undertaken but gave no result, as the whole series proved negative, the monkey not being in the infective condition.

The total number of flies used in the whole group of experiments under consideration in this paper is 1411 males and 1322 females, of which 42 males and 39 females show trypanosomes. Irrespective of sex, the total number is 2733, of which 81 gave a positive result.

From this total must be deducted the starvation experiments and a small group which are not strictly comparable, owing to the feeding having included toad's blood, and also those flies dissected before the 5th day. Concisely the figures stand thus:—

Total	2733 flies;	infected flies,	81.
Toad and starvation experiments.....	234 „ ;	„	17.
Flies dissected between days 1—5 ...	84 „ ;	„	9.

Deducting the last two batches from the total there remain 2415 flies, of which 55 were infected; that is 2·27 per cent. of the flies harboured trypanosomes.

This percentage, *i.e.* 2·27, is naturally not the measure of the infectivity to the fly of any strain (or strains) of trypanosome. It is the percentage of infected individuals produced by allowing 2415 flies to feed at random, in groups, through a period of two and a half months, on a population of nine infected monkeys. Each group receives of course only one or two feeds on the infecting monkey, and is then fed on clean animals.

Certain obscuring features, habitually neglected in dealing with trypanosome infections, must be pointed out in figures handled in this way. The nature of the individual strain must be considered, and the occurrence of negative periods (*i.e.* periods when the vertebrate is not infective to fly) must be duly taken into account. As they stand, the figures above cited have no real meaning. The number of infected individuals obtained by feeding flies at random upon an infected monkey or other vertebrate is neither an index of the infectivity of the strain nor of the potential danger of such an animal at large in a fly area. The percentage, however, of infected individuals produced among flies fed during periods when the blood is infective, gives the index of the virulence of the strain as regards fly. If, on the other hand, batches of, say, 50 or 100 flies were fed on an infected monkey for every day of its life during the course of the disease, the infected glossinæ thus produced would give an index of the infective power of the monkey as a whole.

It is obvious that there are two quite different aspects of the question, and calculations in which they are treated as one must naturally be misleading. In practice it seems usual to neglect this distinction, with the result that there has been a tendency to underestimate the potential transmitting capacity of the fly, and to over-rate its individual idiosyncrasy. Given reasonably favourable conditions of temperature and moisture, it is the strain of trypanosomes and not the fly that within a relatively wide range plays the deciding *rôle* in limiting the number of infected glossina. There is, of course, as has already been mentioned, a serious difficulty in the way of the trypanosome in its attempt to establish itself at all in the glossina, but that must be very nearly constant in all cases.

To consider some of the experiments in greater detail. Monkey 113,

infected by wild flies from the lake-shore, first showed trypanosomes in its blood on July 25, 1911. On August 23, Monkey 113 showed trypanosomes in its blood; 137 flies were fed in groups, 36 of these were treated as a starvation experiment; the whole series proved negative with the exception of one starved cage, which showed one infected fly on the 12th day. On August 24, 45 flies were fed on the same monkey, and there resulted five infected flies, that is, a percentage of 11·1 showed trypanosomes. On August 25, 53 flies were fed, of which two were infected, which is equal to a percentage of 3·7.

Table I.—Monkey 113.

Date.	Condition of blood examined alive.	+ or − laboratory flies.	Number of flies fed.	Percentage of + flies.	Number of experiment.
Aug. 20	No trypanosomes seen in blood ...	No flies fed	—	—	—
,, 21	,, ,, ...	,,	—	—	—
,, 22	,, ,, ...	,,	—	—	—
,, 23	Few ,, ,, ...	−	101	—	38—42
,, 24	Numerous trypanosomes seen in blood	+	45	11·1	44 and 45
,, 25	,, ,,	+	53	3·7	46
,, 26	Very numerous trypanosomes in blood	−	89	—	47—49
,, 27	Very few trypanosomes seen in blood	No flies fed	—	—	—
,, 28	Showed no trypanosomes in blood	− (flies fed both days)	25	—	50
,, 29					
,, 30	Shows few trypanosomes	−	34	—	55 and 56
,, 31	,, ,, ...	+ (box fed on 30th and 31st)	107	1·8	54
Sept. 1	Not examined.				
,, 2					
,, 3					
,, 4	Very few trypanosomes	−	32	—	58
,, 5	,, ,, ...	No flies fed	—	—	—
,, 6	Fair number of trypanosomes; dimorphism of broad and narrow forms very marked	−	49	—	60
,, 7	Fair number of trypanosomes ...	No flies fed	—	—	—
,, 8	Trypanosomes very numerous indeed	+	95	2·1	62 and 64
,, 9	Fair number of trypanosomes, but much fewer than on 8th	No flies fed	—	—	—
,, 10	Not examined.				
,, 11	Few trypanosomes	No flies fed	—	—	—
,, 12	Trypanosomes very numerous indeed	+	70	1·4	68
,, 13	,, ,,	No flies fed	—	—	—
,, 14	,, ,,	,,	—	—	—
,, 15	Blood swarming with trypanosomes	+	20	10·0	75

On August 26, however, 89 flies were fed in three groups on this same monkey, and produced no infected flies at all. The experiments ran con-

currently, and shared the same weather and other external conditions, and were similarly fed. Here, as all through this paper, results of flies dissected before and on the 5th day are excluded. In the experiments just mentioned the flies were dissected at different periods, but all after the 20th day. One is forced to the conclusion that, although the monkey showed trypanosomes on all the four days in question at the time of feeding the flies, the blood was only very slightly infective on the 23rd, probably not at all to fed flies; that on the 24th and 25th it was infective, producing (adding the results for the two days) seven infected individuals out of 98 flies, *i.e.*, 7·1 per cent.; and that it was once more non-infective on the 26th. Under conditions so similar it is impossible to consider a difference of 7·1 per cent. to be due to the individual variations of the two laboratory-hatched sets of flies. The onus of this discrepancy must obviously be borne by the trypanosomes derived from the monkey.

It is interesting to note in this connection the result of microscopic examination of the live blood of this monkey over these and the adjacent days.

For the sake of completeness I add the percentage of infected flies produced over all the boxes containing infected flies obtained from Monkey 113. This includes some results from dates earlier than those of the foregoing table. Starvation results and flies dissected before the fifth day are as usual excluded.

	Flies.	Infected.
Experiment 25	69	2
„ 29	60	1
„ 32	71	1
„ 43	32	3
„ 45	13	2
„ 46	53	2
„ 54	107	2
„ 64	104	2
„ 68	70	1
„ 75	20	2
Total	599	18

The percentage of infected flies is equal to 3 per cent. I do not add the percentage obtained by including all the boxes in which the result was negative. The number so obtained would be a mere numerical curiosity, as, with our present knowledge, it is purely a matter of chance from the point of view of the experimenter, how often he happens to strike a non-infective period. It is obvious that a quite uncontrollable factor is introduced

if the figures are so handled. It becomes clear that although a trypanosome-infection is in a continual state of flux the percentage of plus flies produced over infective periods gives a measure of the virulence of the strain to fly and forms a basis of comparison between different strains.

Another set of experiments bears on this point. Monkey 199 was infected by Dr. Duke by direct injection of blood from a bush-buck which had been infected with *T. gambiense* by laboratory infected flies. The bush-buck had harboured *T. gambiense* for 15 months. This monkey showed infective and non-infective periods in exactly the same way as other infections, but the infective periods gave quite an unusual number of flies harbouring trypanosomes. Thus—

Experiment 71, 13/9/11, 54 flies fed, gave	4	positive,	=	7·4	per cent.
„	70, 14/9/11, 50	„	„	7	„	= 14·0	„
„	74, 15/9/11, 46	„	„	5	„	= 10·8	„

Considering all the figures together, out of 150 flies, 16 showed trypanosomes, that is a percentage of 10·6 per cent.

This relatively very high percentage was also borne out in experiments of Dr. Duke's in which he made use of this monkey and which he kindly permits me to quote. Thus of 188 flies from two experiments the conditions of which admit of comparison with those of Experiments 71, 70, and 74 just cited, 11 were infected, that is 5·8 per cent.

Taking this set of figures with those quoted above, of 338 flies, 27 were infected, which is equal to a percentage of 8 per cent. This is more than double the normal percentage of infected flies produced by the Uganda strain of *T. gambiense* in monkeys. All the conditions being considered, it is impossible to attribute this difference to anything but the strain of trypanosomes.

Besides having a virulent character as regards the production of infected flies as a whole, an individual strain has often a recognisable type or method of development in the glossina. For instance, all the flies fed on Monkey 199 gave very numerous and rapidly developing infections; the trypanosomes reached the proventriculus earlier in the cycle than is usual, and were established in the salivary glands much more promptly than in the case of ordinary cycles. One cage was infective on the 24th day. This difference of character appeared in the flies from Dr. Duke's experiments, as well as in those cited above, and I am indebted to him for the opportunity of examining them. The monkeys infected by fly fed on Monkey 199 showed good infections in the blood (Monkeys 330, 390, and 391), but flies fed on these monkeys gave only an average number of infected flies, *i.e.* 3 per cent., which, however, showed rather sluggish and very slowly developing

infections. Thus, one of the cages fed on one of these monkeys showed an infected fly in which the infection had not yet reached forward beyond the mid-gut on the 22nd day, and another on the 56th day, in which the salivary glands were not yet infected. There was no possible chance of a "pick up" infection in either case. A stray fly, showing a very backward infection, is generally due to having allowed a cage to stay too long on the test animal, that is until after it has produced an infection. A new cycle may then be started in a fly which had escaped on the previous occasion. This point is, I may mention in passing, another argument in favour of the failure of the trypanosomes to establish themselves in the fly being due rather to the flagellates than to any absolute inhibiting quality or condition in the recalcitrant glossina.

Monkey 199 illustrates some particularly important points in regard to the cycle of *T. gambiense* as a whole. It has been shown, by many experiments carried out at Mpumu, that infected buck produce a high percentage of plus flies, but that monkeys infected by means of these flies give in turn only the usual low percentage characteristic of cycles started from monkeys.

The important features are—

1. The long period during which the trypanosomes had been in the buck, namely, 15 months.

2. The infection of 199 by direct injection of the blood from the bush-buck.

3. The large percentage of infective flies yielded by 199 when infective.

4. The loss of this last character when the strain is transmitted by flies to other clean monkeys.

Now, the result of a great deal of biological work during recent years has been to establish the general idea that the function of conjugation or nuclear fusion is not reproduction, but the preservation of the characters of the species as a whole, and the neutralisation of the undue tendency to variation produced by unchecked individual multiplication. In the case of trypanosomes, the individuals run through a relatively very large number of generations in the vertebrate, and in consequence, as is well known, are capable of developing very well marked strains, which might almost be termed varieties. The function of the fly, as is obvious from these experiments, is to sift out these variations of the individual strains, and to produce a fairly even type. There is at present no sound evidence of conjugation in any trypanosome life-cycle so far worked out, and the question must be left unprejudiced. It is a very plausible suggestion that the great and undoubtedly stimulating change of environment that occurs in the alternation of hosts has gradually led to the suppression, and finally

taken the place of conjugation. This hypothesis would explain the labile characters and the extraordinary merging of species in the trypanosome group, but it is obviously open to much criticism on the score of its speculative nature. Further, such a conception stands only so long as no sound evidence of conjugation in any trypanosome cycle is to hand.

In any case it seems clear that the cycle in the fly as a whole, whether conjugation actually occurs or not, has much of the biological significance of that process. This conception is of some importance to workers dealing with laboratory strains passed directly for long periods without reference to the intermediate host.

It might be suggested that the fluctuation in the infectivity of the blood of the vertebrate, on which these experiments have laid particular stress, is due to the coming and going of sexual forms in the peripheral blood. The microscopic appearances in the vertebrate have not as yet been sufficiently closely correlated with the infected periods to permit of any statement as to whether there is a morphological distinction in the individuals at different times.

This paper does not deal with the morphological aspect, but I should like to deprecate the rash use of the terms male and female to describe slender and broad trypanosomes. In *T. gambiense* cycles, in the glossina, very slender elongated forms are produced from the broad forms, as in most other trypanosome life-histories; they are naturally present together in the posterior part of the gut, but the former are destined to pass forwards and form the overwhelming majority of the individuals in the proventriculus and thoracic intestine. The terms male and female are gratuitously misleading until the individuals have been shown to have a sexual connection.

Such a use of language encourages an undue bias in the mind of the observer, and, while I am far from denying the possible and even probable occurrence of a sexual process, it is obviously an unscientific procedure to select the individuals *a priori* by the casual method of analogy, an analogy, moreover, not even drawn from the same class of the protozoa.